THE INHERITANCE OF BLACK–EYED WHITE SPOTTING IN MICE

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BLACK-EYED white varieties of rodents have long been recognized and used as material for genetic investigation.

Cuénot, Morgan and Durham with mice and Castle with guinea-pigs have utilized this particular color variety in breeding experiments. For the most part they are agreed that black-eyed white varieties represent an extreme condition of the ordinary "spotted" or "piebald" series.

Cuénot (1904) in treating the inheritance of spotting concludes that there exists a continuous series of partially pigmented forms extending on the one hand from mice with white on the tail, or with a small white ventral patch, or with small white forehead spot, through a series of decreasingly pigmented forms until the black-eyed white form is reached at the other end of the series. As to a factorial explanation for the phenomena observed in the inheritance of spotting, Cuénot feels that there are numerous stages of the spotted condition (P) which he designates by p^1, p^2, p^3, p^4 as progressively whiter forms are considered. He believes, however, that the details of spotting are not represented in the germ cell. He further mentions the failure to obtain any particular stage of spotting in a true breeding condition. Selection of nearly solid-colored forms has enabled him to obtain animals with greatly increased white areas.

Durham (1908) has obtained some evidence for two different types of spotting, one recessive to solid-coated forms and one dominant to them. She has reported several crosses which I have considered in more or less detail in another paper (Little, 1914). None of the crosses presented by her can be considered as critical tests of the presence of two distinct spotting factors. Morgan (1909),

who has worked with the same types as Durham, feels uncertain as to the real significance of black-eyed whites and as to the occurrence of a distinct factor for dominant spotting.   This uncertainty I also felt and have tried to show further reasons for not considering Miss Durham's work as establishing the existence of a dominant spotting factor.

Castle (1905) has found that in guinea-pigs black-eyed whites behave in inheritance in much the same way that the same type of mouse behaves, namely that black-eyed whites do not breed true but give, when crossed *inter se,* a whole range of spotted forms in addition to some like themselves.

One can by selection progress in either direction through a series of spotted forms, decreasing or increasing the number and extent of pigment patches.   Great difficulty, however, was encountered in trying to fix the color pattern at any particular stage in the series.   Up to the present time this has not been proved possible.

Experimental

In the early winter of 1913 Dr. Castle obtained from a fancier in England two pairs of black-eyed white mice. These he kindly handed over to me for investigation. From the outset the progeny of these mice proved to be extremely healthy and vigorous.

1. *Black-eyed White Crossed Inter Se*

This cross gave two distinct classes of young, black-eyed white and ''piebald.''   The distinction between the two classes can best be shown by the tabulation of their progeny on the basis of the amount of dorsal pigmentation they possess.   I have for some time estimated the per cent. of the dorsal surface pigmented in the case of all spotted animals recorded.   This gives a basis for classification which, though it may at first glance seem to inexact, nevertheless has been shown by comparing the
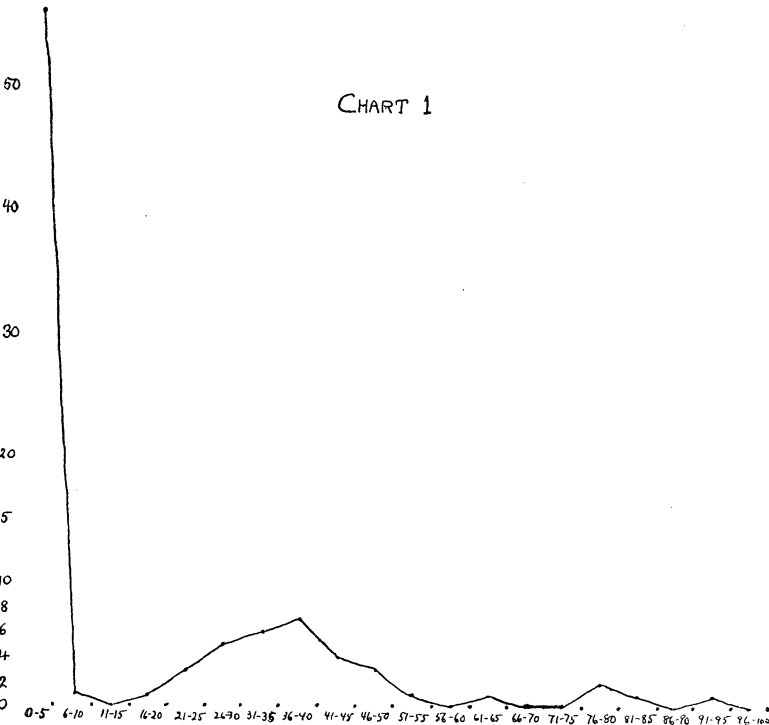
estimates of two or more investigators on any one animal to be surprisingly exact and fully as satisfactory as any other system of grading.

TABLE I.

Type of Cross	Per Cent of Dorsal Pigmentation																			
	0–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65	66–70	71–75	76–80	81–85	86–90	91–95	96–100
Black-eyed white *inter se*	56	1	0	1	3	5	6	7	4	3	1	0	1	0	0	2	1	0	1	0
Black-eyed white × piebald	105	0	0	1	1	12	8	11	5	6	4	4	1	3	4	9	4	9	3	0
Piebald × piebald	0	0	0	0	0	4	6	7	13	6	2	9	4	6	2	5	8	15	2	0
Total	161	1	0	2	4	21	20	25	22	15	7	13	6	9	6	16	13	24	6	0

From Chart I it will be seen that 44 of the 75 young obtained fall in the class between 0 and 5 per cent. of dorsal pigmentation. These are the black-eyed whites.

CHART 1

The remaining 31 young are more or less scattered along
the range of ''piebald'' forms.   The gap between the two
classes is a considerable one and is certainly significant.

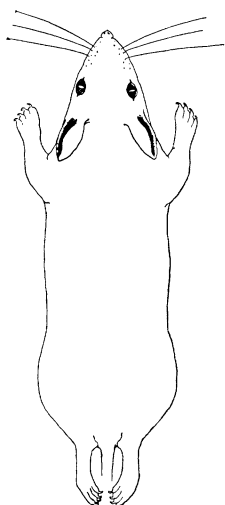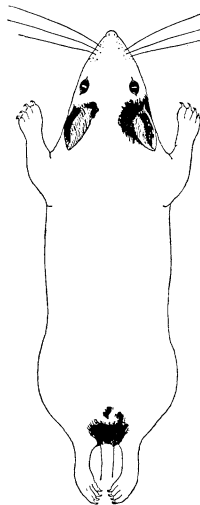FIG. 1   FIG. 2

Figs. 1–4 are diagrammatic and are intended to show
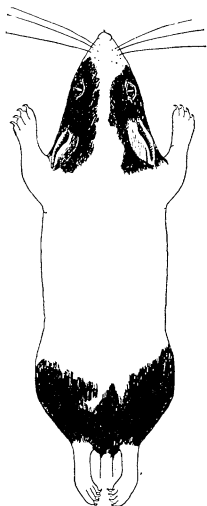the two groups of spotted animals.   Figs. 1 and 2 show

FIG. 3   FIG. 4

the common range of variation within the black-eyed
white type and Figs. 3 and 4 the same for the "piebald"
type.

2. *Black-eyed White* $\times$ *Piebald*

This mating brought out two interesting facts. *First,*
all black-eyed whites behaved in essentially the same way,
approximately an equal number of black-eyed white and
piebald young being produced. *Second,* the same dis-
tinctness between the two types held good, as will be seen
from the chart given below (solid line).

3. *Piebald* $\times$ *Piebald*

Piebald animals from black-eyed white parents and
from the cross of piebald $\times$ black-eyed white were mated
*inter se.   They produced only piebald young, 93 in
number.*

The distribution of these young according to the degree
of dorsal pigmentation they possessed is shown by Chart
2 (dotted line).

It will be noticed that there is no approach to the black-
eyed white condition (0–5 per cent.).   There are also in-
dications of two main modal points, one at 41–50 per cent.
and one at 80–90 per cent. A complete curve formed from
the sum of all piebald animals included in Table I, is
given in Chart 2 (broken line).

This further emphasizes the bi-modal nature of the
curve in the case of piebald mice and makes it seem likely
that there are two genetically distinct grades of this
variety.   It is hoped that opportunity will arise in the
future to investigate this point more accurately.

4. *Discussion*

From the three types of matings given above the fol-
lowing facts may be deduced: (1) The inheritance of the
characters in question is alternative, not blending in
nature; (2) black-eyed white is epistatic to ordinary pie-
bald spotting.

CHART 2

BLACK- EYED WHITE X PIEBALD (SOLID LINE) ―
PIEBALD X PIEBALD (DOTTED LINE)......
COMBINATION OF ALL PIEBALD (BROKEN LINE)―.―

The behavior of black-eyed whites in crosses 1 and 2, Table I indicates that they are always heterozygous dominants and that they can not, therefore, be obtained in a condition to "breed true."

With this in mind it is interesting to calculate the expected ratio when black-eyed whites are crossed *inter se*. If black-eyed white is due primarily to a dominant factor

which obeys the ordinary laws of mendelian inheritance, we should expect that black-eyed whites would be obtained of two genetic types, homozygous and heterozygous. If now black-eyed whites were mated together at random, the matings should be either (1) DD × DD, (2) DD × DR or (3) DR × DR. In the case of (1) and (2) only black-eyed white young should be produced, while type (3) should give approximately 3 black-eyed whites to one piebald. Random matings would therefore produce a ratio of black-eyed whites to piebalds considerably in excess of 3 : 1.

If, on the other hand, the DD form of black-eyed white mice behaves in a fashion similar to the homozygous yellow mice, failing to develop, we should expect a ratio of 2 black-eyed whites to one piebald young, no matter what the origin of the black-eyed white parents might be, whenever two black-eyed whites are bred together.

The results are as follows:

Black-Eyed White × Black-Eyed White

	Black-eyed White	Piebald
Observed	57	39
Expected 2:1 ratio	64	32
Expected 3:1 ratio	72	24

When one realizes that the ratio in one case should be considerably *higher* than 3 : 1, it seems that the results indicate a 2 : 1 ratio and the heterozygous nature of black-eyed whites.

To further test this hypothesis individual tests of twenty-one black-eyed whites coming from black-eyed white parents were made by crossing with piebald animals. If the DD combination is possible, approximately seven of the twenty-one tested should be of that constitution. All of them, however, proved to be heterozygous. While the numbers should be supplemented by further tests, they are certainly sufficient to serve as a basis for a tentative conclusion that black-eyed white mice are always heterozygous.

The numbers from the cross of piebald × black-eyed white are more extensive and closely approximate a 1:1 ratio. The numbers obtained are 105 black-eyed whites and 102 piebald, while the 103 of each would have been exactly an equality ratio.

The behavior of the piebald animals when crossed *inter se* is exactly what would be expected if piebald was hypostatic to black-eyed white and distinct from it in inheritance.

The next question to be considered is the relation of black-eyed white to ''self'' or solid coat, in inheritance.

RELATION OF BLACK-EYED WHITE TO SELF

A preliminary investigation of this question has been made. The ''self'' race used was really technically not a ''self'' but genetically it carried neither the black-eyed white nor piebald spotting factors. Somatically the self race used was a ''blaze'' race of the type which I have previously put on record. Further crosses which I have made between black-eyed whites and true selfs have shown, even in early stages, clear evidence that the behavior of the blaze and true self races is directly comparable.

1. *"Self" × Black-eyed White*

The F_1 generation produced by crossing self (blaze F6B) animals with black-eyed whites consists of two very distinct forms. These have been produced in a ratio of 50 Type ''A'' to 47 Type ''B.'' The first of these, Type ''A,'' is shown in Fig. 5. While the percentage of dorsal pigmentation of this type is subject to some variation (see table), it will be noticed that they are ordinarily between 80 and 90 per cent. colored. The spots of color

Type "A"	36–40	41–45	46–50	51–55	56–60	61–65	66–70	71–75	76–80	81–85	86–90	91–95	96–100
Black-eyed white × self (blaze)	0	1	2	0	1	1	2	0	9	15	10	8	1

appear to have slightly more irregular and less clearly
defined outlines than do those of the ordinary piebald
mice and many of the spots are distinctly smaller in size
(compare Figs. 3, 4 and 5).   Just how much of this ap-

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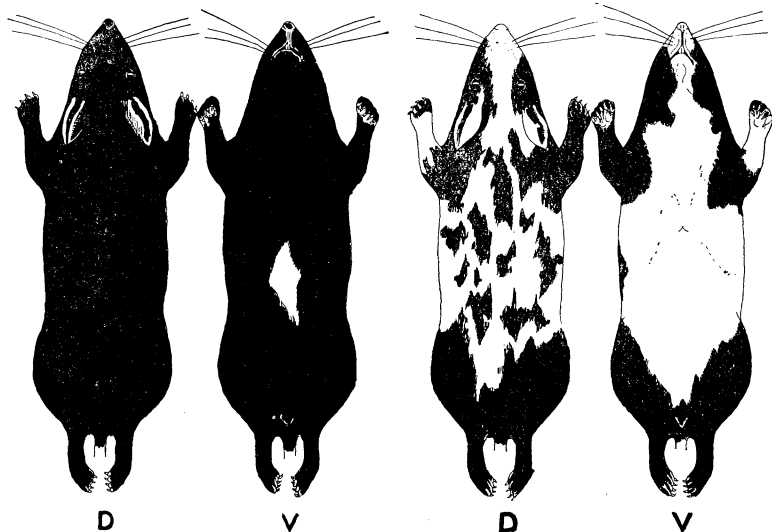
FIG. 5   FIG. 6

pearance is due to true genetic difference between the two
types of spotting is of course problematical and must
remain so until a larger mass of data is available.

Concerning class ''B'' (Fig. 6) little need be said save
that they appear in every way identical with heterozy-
gotes ordinarily obtained in a cross between ''self'' and
''piebald'' animals.   They vary from entirely solid colored
animals to those having approximately 20 per cent. of the
ventral surface white.   They may be tabulated as follows:

	Per Cent of White on Ventral Surface						
	0	1–5	6–10	11–15	16–20	21–25	26–30
Type ''B''	5	28	8	3	2	1	0

2. *Type ''A'' Animals Crossed Inter Se*

Type ''A'' animals obtained in F_1 are distinctly ''spot-
ted.''   They have a clearly discernible amount of white

and are not in the least like heterozygous "selfs" of any recorded type. When crossed together they give three somatically distinct classes of young, "self," "piebald" or like class "A," and black-eyed white. The numbers obtained are 15 "self," 31 spotted (piebald or like class "A") and 11 black-eyed whites.

3. *Type "A" × Piebald*

To test them further type "A," animals of this class were crossed with homozygous piebald mice extracted from the black-eyed white crosses. Again three general classes of young were obtained as follows: 45 "self," 54 spotted (piebald or like type "A") and 29 black-eyed whites.

4. *Type "B" × Piebald*

To compare the behavior of types "A" and "B" this cross was made. Only two classes of young resulted as follows: 82 class "B" and 78 piebald. No black-eyed whites were obtained.

Discussion

The question now arising is whether the factors for self, black-eyed white, and piebald are allelomorphic or independent in inheritance.

From the nature of the F_1 generation it is certain that the black-eyed white animals are forming two kinds of gametes in respect to their spotting factors.

If now the conditions "self" coat, "black-eyed white" and "piebald" are all related as members of a system of triple allelomorphs, we can express the cross as follows:

$$S = \text{self factor.}$$
$$W = \text{black-eyed white factor.}$$
$$sp = \text{piebald factor.}$$

Then

$$S\ S = \text{self} \times W sp = \text{black-eyed white}$$

gametes   S   W
              sp

F_1 Generation   S W = Type A, Fig. 5
                          S sp = Type B, Fig. 6

If now animals of Type A are bred *inter se* we should expect

    S | W × S W
  1 SS = self
  2 SW = like Type "A"
  1 WW = (not formed because homozygous)

The one WW individual could not be formed since by experiment it has been shown that W can exist in only one of the two gametes forming a zygote. When W meets S, an animal like Class A is produced, when it meets sp a black-eyed white results.

The expectation therefore is that, if a system of triple allelomorphs is operative here, we should have *no black-eyed whites formed from mating together class "A" animals.*

The result of this mating quickly settles the above hypothesis for 15 "self" colored, 31 spotted (like or nearly like Type "A"), and 11 black-eyed whites have been obtained.

It is clear, therefore, that "black-eyed white" depends upon a factor which is at least partly independent of that producing "piebald" spotting. Let us suppose that this is the case and that "black-eyed whites" always carry piebald in all of their gametes and an epistatic inhibiting or restrictive factor producing increased whiteness in one half their gametes. If W equals restrictor and w its absence and sp equals the factor for piebald spotting, all black-eyed whites will be Wwspsp, in zygotic formula and will form two sorts of gametes, Wsp and wsp.

This will account for the results in mating black-eyed whites *inter se* due to the failure of the WWspsp zygote to continue its development because of the double dose of W.

If now black-eyed whites Wwspsp are crossed with selfs wwSS, two classes of F_1 zygotes will result, WwSsp and wwSsp. The former will produce a new zygotic combi-

nation really differing from the black-eyed whites in the
substitution of a "self" bearing gamete for a "piebald"
one in the zygotic formula.   The result is an animal like
Type "A," Fig. 5; Type "B," Fig. 6 shows the other F_1
type which is entirely free from the W factor and which
is merely a heterozygote between "self" and "piebald."

If class "A" animals are crossed *inter se* we should on
this new hypothesis expect the following results.

1 WWSS .... not developed
2 WwSS .... very dark spotted
2 WWSsp .... not developed
4 WwSsp .... like parents (type "A")
1 WWspsp ...... not developed
2 Wwspsp .... black-eyed white
1 wwSSp .... "self"
2 wwSsp .... "self" or "self" with white ventral patch (type "B")
1 wwspsp .... "piebald"

Four of the 16 zygotes in F_2 would have two doses of
W and would not develop.   Of the remaining 12, seven
would have some degree of white spotting depending upon
whether they were WwSS, WwSsp or wwspsp in formula;
three would be "solid" colored or like type "B" of F_1
and two would be black-eyed whites.

On this hypothesis the F_2 generation would be as fol-
lows:

	Observed	Expected
Solid	15	15
Spotted	31	35
Black-eyed whites	11	10
	57	60

A further test of the nature of type "A" is possible.
If they are bred to piebald animals, four classes of young
should result as follows.

WwSsp .... like class "A"
Wwspsp .... black-eyed whites
wwSsp .... solid colored
wwspsp .... piebald

Lumping together the WwSsp and the wwspsp animals

we should have 2 spotted, 1 black-eyed white and 1 self. The results are as follows:

	Observed	Expected
Spotted	54	64
Self	45	32
Black-eyed white	29	32
	128	128

Whether the excess of "self" animals is significant is, of course, a question to be borne in mind but it is extremely doubtful whether it is due to anything more than a chance deviation.

Type "B" animals have, upon mating with "piebald" individuals, given very close to the expected ratio of 1 type "B" to 1 "piebald." The exact numbers are 82:78; expected ratio 80:80.

Is Black-eyed White in Mice an Allelomorph of Albinism?

The experiments of Castle and Wright have shown that a dark red-eyed variety of guinea-pig exists which is an allelomorph of dilute pigmentation and of albinism. This possibility in the case of mice is eliminated by crossing black-eyed white with albino, when on the supposition that the condition found in guinea-pigs holds true in mice all the young should be either black-eyed white, albino or dilute pigmented. Actually there were obtained from a single mating of this sort five young, all intensely pigmented, two blacks and three browns; thereby eliminating the possibility that black-eyed white, in mice, is an allelomorph in the albino series.

Conclusions

The fact that black-eyed white spotting in mice appears to be due to a factor independent of and supplementary to the factor for "piebald" spotting leads to interesting speculation as to the nature of spotting and

indicates that spotting in mice is dependent upon more than one pair of clear-cut mendelizing factors. Modifying factors which may be more or less difficult to analyze but which nevertheless are certainly present, contribute to the extent of variation in spotted races.

"Blaze" or forehead spotting is apparently independent of ordinary "piebald" spotting, as I shall hope to show in a future paper; "black-eyed white" is primarily due to an independent genetic factor and "piebald" makes a third independent type. If now in the "piebald" stock there exist at least two genetic races as are indicated by the curve of all piebald animals obtained in the "black-eyed white" crosses, the condition is still further complicated. At all events one can truthfully say that the distribution of pigment occurring as it does along a series from "self" colored to "black-eyed white" animals, offers a field for the activity of many mendelizing factors. There is no *a priori* reason why this should not be true, there are many experimental reasons steadily increasing why it appears to *be* true.

Spotting in rodents is tempting as genetic material because of the clear patterns and contrast between colored and white areas. It is, however, *as a character* extremely sensitive to minute quantitative and qualitative changes and its apparent genetic simplicity is a snare and a delusion.

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